Roles of Hedgehog pathway components and retinoic acid signalling in specifying zebrafish ventral spinal cord neurons.

Journal: Development

Publication Year: 2011

Authors: Samantha England, Manuel F Batista, John K Mich, James K Chen, Katharine E Lewis

PubMed link: 22069186

Funding Grants: Stanford CIRM Training Program

Public Summary:
The central nervous system (CNS) has resident neural stem cells that maintain self-renewal and multipotency well into adulthood, and can respond to CNS injury with a limited ability to repopulate lesions with newborn cells. As a result, future stem cell therapies (by either activating endogenous neural stem cells, or supplying exogenous neural stem cells) will hopefully become widespread for a number of CNS pathologies, such as spinal cord injury and neurodegenerative diseases such as Parkinson's. However, our current incomplete understanding of neural stem cell biology limits our ability to take full advantage of their regenerative potential for therapeutic purposes. For example, we need to know what key signals cause the formation of neural subtype identities ("fates") and how these signals are integrated, in order to be able to manipulate specific signals to control neuronal fates. To learn which signals instruct undifferentiated neural tissue to acquire mature neuronal fates, we sought to remove specific signals from the zebrafish embryonic neural tube and determine the effects on neuronal fates. Zebrafish embryos are useful for these studies because they are easy to manipulate genetically and pharmacologically, and their spinal cord neuronal subtypes are found in stereotyped positions that are easy to identify. One family of signals that is known to be important for acquisition of neuronal fates in the ventral CNS is the Hedgehog family of secreted proteins, but technical issues had thus far prevented us from knowing the exact role of Hedgehog signaling in CNS fate determination. In order to precisely define the role of Hedgehog signaling we generated MZsmo mutant embryos, which lack a transmembrane protein which is required for all cellular responses to Hedgehog signals. As we predicted, in these embryos most of the ventral-most cells in the spinal cord no longer formed, but unexpectedly a small number still remained. In addition, more dorsal neuronal cell types in the ventral spinal cord were unaffected in number (another unexpected result) although they were found more ventrally than normal. To investigate which other factors might account for the ventral spinal cord neurons that persist in MZsmo embryos we eliminated several factors in addition to Hedgehog signaling. We found that activity of the Gli transcription factors is needed for the few remaining ventral-most neurons to form, but not for the more dorsal cell types to form. We also found that the chemical retinoic acid was important for the correct numbers of neurons to form across all domains, but for most cell types, some cells still form, even in the absence of Hedgehog and Retinoic Acid signaling. As a result, the identity of the important factor(s) that instruct the formation of the more dorsal ventral spinal cord cell types remains an intriguing unsolved question. Incorporating this knowledge into an integrative view of the factors that control neural cell fate determination will be essential for harnessing the promise of neural stem cell-based therapies.

Scientific Abstract:
In mouse, Hedgehog (Hh) signalling is required for most ventral spinal neurons to form. Here, we analyse the spinal cord phenotype of zebrafish maternal-zygotic smoothened (MZsmo) mutants that completely lack Hh signalling. We find that most V3 domain cells and motoneurons are lost, whereas medial floorplate still develops normally and V2, V1 and V0v cells form in normal numbers. This phenotype resembles that of mice that lack both Hh signalling and Gli repressor activity. Ventral spinal cord progenitor domain transcription factors are not expressed at 24 hpf in zebrafish MZsmo mutants. However, pMN, p2 and p1 domain markers are expressed at early somitogenesis stages in these mutants. This suggests that Gli repressor activity does not extend into zebrafish ventral spinal cord at these stages, even in the absence of Hh signalling. Consistent with this, ectopic expression of Gli3R represses ventral progenitor domain expression at these early stages and knocking down Gli repressor activity rescues later expression. We investigated whether retinoic acid (RA) signalling specifies ventral spinal neurons in the absence of Hh signalling. The results suggest that RA is required for the correct number of many different spinal neurons to form. This is probably mediated, in part, by an effect on cell proliferation. However, V0v, V1 and V2 cells are still present, even in the absence of both Hh and RA signalling. We demonstrate that Gli1 has a Hh-independent role in specifying most of the remaining motoneurons and V3 domain cells in embryos that lack Hh signalling, but removal

of Gli1 activity does not affect more dorsal neurons.
